

International Application No.: PCT/US2004/042701
International Filing Date.: December 17, 2004
First Preliminary Amendment dated June 16, 2006

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-369. (Canceled)

370. (New) A method for conjugating a peptide immunogen via a reactive group of an amino acid residue of the peptide immunogen to a protein/polypeptide carrier having one or more functional groups, the method comprising the steps of:

- (a) derivatizing one or more of the functional groups of the protein/polypeptide carrier or optionally to a polypeptide linker attached to the protein/polypeptide carrier to generate a derivatized carrier with reactive sites;
- (b) reacting the derivatized protein/polypeptide carrier of step (a) with a reactive group of an amino acid of the peptide immunogen under reaction conditions such that the peptide immunogen is conjugated to the derivatized protein/polypeptide carrier via the functional groups; and
- (c) further reacting the conjugate with a capping reagent to inactive free, reactive unreacted functional groups on the derivatized protein/polypeptide carrier, thereby preserving the functionality of the carrier, such that it retains its ability to elicit the desired immune responses against the peptide immunogen that would otherwise not occur without a carrier.

371. (New) The method of claim 370, wherein the protein/polypeptide carrier is selected from the group consisting of human serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin, cholera toxin mutants

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with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

372. (New) The method of claim 371, wherein the protein/polypeptide carrier is CRM₁₉₇.

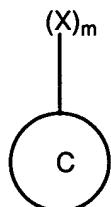
373. (New) The method of claim 370, wherein the peptide immunogen is selected from the group consisting of a bacterial protein, a viral protein, and a eukaryotic protein.

374. (New) The method of claim 370, wherein the functional group of one or more amino acid molecules of the protein/polypeptide carrier or of the optionally attached polypeptide linker is derivatized using a cross-linking reagent.

375. (New) The method of claim 374, wherein the protein/polypeptide carrier is reacted with a haloacetylating agent.

376. (New) The method of claim 370, wherein the capping reagent that is used to inactivate free reactive, functional groups on the activated protein/polypeptide carrier is selected from the reagent group consisting of cysteamine, N-acetylcysteamine, ethanolamine, sodium hydroxide, sodium carbonate, ammonium bicarbonate and ammonia.

377. (New) A method for conjugating a peptide immunogen to a protein/polypeptide carrier having the structure:

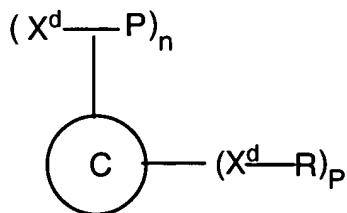


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wherein,

C is a protein/polypeptide carrier and X is a derivatizable functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to the protein/polypeptide carrier, and wherein m is an integer greater than 0, but less than or equal to 85, the method comprising the steps of:

- (a) derivatizing one or more of the functional groups of the protein/polypeptide carrier or of the optionally attached linker molecule to generate a derivatized molecule with reactive sites;
- (b) reacting the derivatized protein/polypeptide carrier of step (a) with a reactive group of an amino acid residue of the peptide immunogen to form a covalently coupled peptide immunogen-protein/polypeptide carrier conjugate; and
- (c) further reacting the said conjugate with a capping reagent to inactivate the free reactive functional groups on the activated protein/polypeptide carrier, such that the capped groups are not free to react with other molecules, thereby preserving the functionality of the carrier, such that it retains its ability to elicit the desired immune responses against the peptide immunogen that would otherwise not occur without a carrier, so as to generate a capped peptide immunogen-protein/polypeptide carrier conjugate having the formula:



wherein,

C is the protein/polypeptide carrier and X^d is a derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to the protein/polypeptide carrier, and, wherein,

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P is a peptide immunogen molecule covalently attached to the derivatized functional group of the amino acid residue of the protein carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier,

R is a capping molecule covalently attached to the derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier,

n is an integer greater than 0, but less than or equal to 85, and

p is an integer greater than 0, but less than 85.

378. (New) The method of claim 377, wherein the protein/polypeptide carrier is selected from the group consisting of serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin, cholera toxin mutants with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

379. (New) The method of claim 378, wherein the protein/polypeptide carrier is CRM₁₉₇.

380. (New) The method of claim 377, wherein the peptide immunogen is selected from a bacterial protein, a viral protein, and a eukaryotic protein.

381. (New) The method of claim 377, wherein the functional group of one or more amino acid molecules of the protein/polypeptide carrier or of the optionally attached polypeptide linker is derivatized using a cross-linking reagent.

382. (New) The method of claim 381, wherein the protein/polypeptide carrier is reacted with a haloacetylating agent.

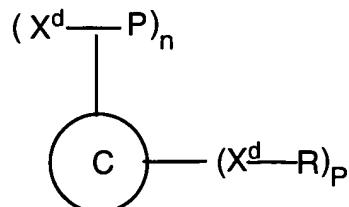
383. (New) The method of claim 377, wherein the capping reagent that is used to inactivate free reactive, functional groups of the activated protein/polypeptide carrier is selected from the reagent group consisting of cysteamine, *N*-acetylcysteamine, ethanolamine, sodium hydroxide, sodium carbonate, ammonium bicarbonate and ammonia.

384. (New) A peptide immunogen-protein/polypeptide carrier conjugate wherein the protein/polypeptide carrier has the formula:



wherein,

C is a protein/polypeptide carrier and X is a derivatizable functional group of an amino acid residue on the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to the protein/polypeptide carrier, and, wherein m is an integer greater than 0, but less than or equal to 85, and wherein the peptide immunogen-protein/polypeptide carrier conjugate has the formula:



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wherein,

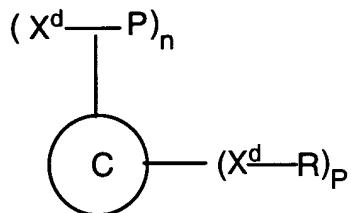
C is the protein/polypeptide carrier and X^d is a derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to the protein/polypeptide carrier, and, wherein, P is a peptide immunogen molecule covalently attached to the derivatized functional group of the amino acid residue of the protein carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier, R is a capping molecule covalently attached to the derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier, thereby preserving the functionality of the carrier such that it retains its ability to elicit the desired immune responses against the peptide immunogen that would otherwise not occur without a carrier., n is an integer greater than 0, but less than or equal to 85, and p is an integer greater than 0, but less than 85.

385. (New) The conjugate of claim 384, wherein the protein/polypeptide carrier is selected from the group consisting of serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin, cholera toxin mutants with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

386. (New) The conjugate of claim 385, wherein the protein/polypeptide carrier is CRM₁₉₇.

387. (New) The conjugate of claim 384, wherein the peptide immunogen is selected from the group consisting of a bacterial protein, a viral protein, and a eukaryotic protein.

388. (New) A peptide immunogen-protein/polypeptide carrier conjugate generated according to the method of claim 377 and having the formula:



wherein,

C is the protein/polypeptide carrier and X^d is a derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to the protein/polypeptide carrier, and, wherein, P is a peptide immunogen molecule covalently attached to the derivatized functional group of the amino acid residue of the protein carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier, R is a capping molecule covalently attached to the derivatized functional group of an amino acid residue of the protein/polypeptide carrier or optionally of an amino acid residue of a peptide linker covalently attached to a protein/polypeptide carrier, which preserves the functionality of the carrier, such that it retains its ability to elicit the desired immune responses against the peptide immunogen that would otherwise not occur without a carrier, n is an integer greater than 0, but less than or equal to 85, and p is an integer greater than 0, but less than 85.

389. (New) The conjugate of claim 388, wherein the protein/polypeptide carrier is selected from the group consisting of human serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin,

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cholera toxin mutants with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

390. (New) The conjugate of claim 389, wherein the protein/polypeptide carrier is CRM₁₉₇.

391. (New) The conjugate of claim 388, wherein the peptide immunogen is selected from the group consisting of a bacterial protein, a viral protein, a fungal protein, a parasite protein and a eukaryotic protein.

392. (New) An immunogenic composition, comprising a conjugate of a peptide immunogen with a protein/polypeptide carrier generated by the method of claim 377, together with one or more pharmaceutically acceptable excipients, diluents, and /or adjuvants.

393. (New) The immunogenic composition of claim 392, wherein the protein/polypeptide carrier is selected from the group consisting of human serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin, cholera toxin mutants with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

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394. (New) The immunogenic composition of claim 393, wherein the protein/polypeptide carrier is CRM₁₉₇.

395. (New) The immunogenic composition of claim 392, wherein the peptide immunogen is selected from the group consisting of a bacterial protein, a viral protein, a fungal protein, a parasite protein, and a eukaryotic protein.

396. (New) The immunogenic composition of claim 392, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529 SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL™ (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, STIMULON™ QS-21, a pertussis toxin (PT), an *E.coli* heat-labile toxin (LT), IL-1 α, IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon-α, interferon-β, interferon-γ, G-CSF, TNF-α and TNF-β.

397. (New) A method for inducing an immune response in a mammalian subject, which comprises administering an effective amount of the immunogenic composition of claim 392 to the subject.

398. (New) The method of claim 397, wherein the protein/polypeptide carrier is selected from the group consisting of human serum albumin, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, influenza hemagglutinin, PADRE polypeptide, malaria circumsporozite (CS) protein, hepatitis B surface antigen (HBsAg₁₉₋₂₈), Heat Shock Protein (HSP) 65, *Mycobacterium tuberculosis*, cholera toxin, cholera toxin mutants with reduced toxicity, diphtheria toxin, CRM₁₉₇ protein that is cross-reactive with diphtheria toxin, recombinant Streptococcal C5a peptidase, *Streptococcus pyogenes* ORF1224, *Streptococcus pyogenes* ORF1664, *Streptococcus pyogenes* ORF2452, *Chlamydia pneumoniae* ORF T367, *Chlamydia pneumoniae* ORF T858, Tetanus toxoid, HIV gp120 T1, components recognizing microbial surface adhesive matrix molecules (MSCRAMMS), growth factors, hormones, cytokines and chemokines.

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399. (New) The method of claim 398, wherein the protein/polypeptide carrier is CRM₁₉₇.

400. (New) The method of claim 397, wherein the peptide immunogen is selected from the group consisting of a bacterial protein, a viral protein, and a eukaryotic protein.

401. (New) The method of claim 397, further comprising administering one or more adjuvants selected from the group consisting of GM-CSF, 529 SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL™ (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, STIMULON™ QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1 α, IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon-α, interferon-β, interferon-γ, G-CSF, TNF-α and TNF-β.